

Controlling Of UTI Pathogens Using Commelina Benghalensis Mediated Zinc Oxide Nanoparticles

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Abstract

Introduction: Utilizing commelina benghalensis mediated zinc oxide nanoparticles for controlling pathogens causing urinary tract infections (UTI). Zinc oxide nanoparticles have antioxidant and antimicrobial properties which makes them useful component to use against UTI pathogens

Aim: To synthesize zinc oxide nanoparticles in eco friendly environments and to evaluate the antibiotic and antibacterial effects of zinc oxide nanoparticles mediated by commelina benghalensis plant extract.

Materials and methods: The plant extract is prepared by utilizing commelina benghalensis as a component. The zinc oxide nanoparticles are synthesized using zinc sulfate and distilled water. The antibacterial and inhibitory effect of nanoparticles were evaluated by agar well diffusion technique.

Results: The results suggest that *C. benghalensis* (ZnO NPs) has antibacterial properties against these UTI pathogens, with increasing effectiveness at high concentrations. The "Standard" category shows much larger zones of inhibition for *S. aureus* and *E. coli* (both around 40 mm), while *Klebsiella* sp. has a lower value (about 15 mm).

Conclusion: This study aims to demonstrate the effects of commelina benghalensis mediated zinc oxide nanoparticles against UTI pathogens. Further research and comparison is needed for evaluating antibacterial effects of Zinc oxide nanoparticles. Future research could further explore the specific mechanisms by which these nanoparticles inhibit bacterial growth, potentially leading to new treatments or preventive measures for UTIs.

Key Words: Zinc Oxide, Nanoparticles, commelina benghalensis, antimicrobial, UTI

INTRODUCTION

Urinary tract infections (UTI) are one of the most common and serious bacterial infections encountered by paediatricians and primary care physicians. Although the diagnosis and management of UTI appear simplistic, they remain among the most contentious issues in paediatrics. In part, UTI controversies stem from the absence of classic clinical symptoms, inappropriate urine specimen collection, modified urinary tract imaging recommendations, and diverse treatment and prevention strategies. Recently published guidelines and large clinical trials have attempted to clarify UTI diagnostic and management strategies. In this manuscript, we review the diagnosis and management of acute and recurrent UTI in the paediatric and adolescent populations. (Chandra *et al.*, 2020) ('Green synthesised zinc oxide nanoparticles reveal potent in vivo and in vitro antibacterial efficacy against *Proteus mirabilis* isolates', 2023)

Uropathogens carry multiple virulence factors involved in the pathophysiology of UTIs. These virulence factors are involved in invasion and colonization, as well as in mediating the subversion of host defences. Knowledge about the mechanism of action of these virulence factors is being used to develop new therapeutics against UTIs. Therapies that are currently in the initial stages of development include vaccines targeting bacterial factors that are essential for initial attachment and disease progression (such as adhesins, toxins, proteases and siderophores), and small-molecule inhibitors that prevent adhesin–receptor interactions. (Camara *et al.*, 2021)

The incidence of antibiotic resistant urinary tract infections (UTIs) in children is increasing. In particular, there is growing concern about the emergence of multidrug resistant (MDR) UTIs caused by Enterobacteriaceae. The purpose of this study is to describe the incidence, clinical characteristics and risk factors for MDR UTIs presenting to the pediatric emergency department. (Institute of Medicine, Board on Global Health and Forum on Microbial Threats, 2010)

A vast array of biotechnology applications has been made possible in recent decades by the development of innovative methods for creating nanoformulations, or nanocarriers, that effectively carry medicinal

molecules [Harshitha et al. 2024]. To lessen the negative effects of conventional medicines, smart nanostructured materials can deliver medications to the target locations in a (spatial/temporal) regulated manner and at a lower dosage frequency. Specifically, they enable the resolution of the primary and most significant problems associated with traditional pharmaceutical therapies, including inadequate bioavailability, fast clearance, unpredictable drug release, and nonspecific distribution [Maheswaran et al. 2024][Palani et al.]. A sensitive decrease in toxicity and/or unfavorable responses is the overall result. But even with the amazing advancements in recent techniques, the majority of nanocarriers' actions are linked to a number of unfavorable side effects that reduce. (Elango, Okram and Mathanmohun, 2023)(Ullah and Lim, 2022)

Zinc oxide nanoparticles (ZnO NPs) are a recognized safe substance by the FDA, high surfactant reactivity, and high antimicrobial activity due to a variety of mechanisms such as the production of reactive oxygen species (ROS), the release of Zn^{2+} ions, and disruption of membranes. The ability to overcome resistance through this multimodel action means ZnO NPs are highly effective against both Gram positive and Gram negative bacteria (including UTI pathogens). There continue to be more studies about the benefits of plant mediated green synthesis of ZnO NPs whereby the phytochemicals are used as reducing and stabilization agents, providing more bioactivity and an eco-friendly overall strategy. Given these points, the current study also provides green synthesized ZnO nanoparticles using the resources of *Commelina benghalensis* and their efficacy against UTI pathogens. (Ramachandran et al. 2024) Foodborne illness is becoming a serious worldwide public health threat. The World Health Organization (WHO) estimates that each year, foodborne diseases affect more than 30% of the population in developed countries. Eating food contaminated with foodborne pathogens, such as bacteria, fungus, viruses, and toxins, is the primary cause of foodborne illness in humans. Food, particularly improperly produced food, can become contaminated during pre-harvesting, post-harvesting, processing, shipping, handling, or preparation [1]. *Salmonella*, *Klebsiella*, *Staphylococcus*, and *Bacillus* species are a few frequent pathogens detected in food [2], [3], and [4]. An essential component of public health is pathogen control, particularly when it comes to foodborne illnesses [5, 6]. Economic factors have reduced the use of antibiotics and other currently used disease control measures. (World Health Organization, 2008)(Fink, 2019)

MATERIALS AND METHODS

Preparation of plant extract :

Preparing plant extracts for nanoparticles involves several steps to extract bioactive compounds that can be used in nanoparticle synthesis. Choose plant components (leaves) that are known to contain bioactive substances that are appropriate for the creation of nanoparticles, such as flavonoids and polyphenols. To prepare plant extract , 1 gram of plant powder is mixed with 100ml of distilled water. The solution was heated in a heating mantle for 15-20 min for better extraction of the required compounds. After boiling for the given time , the mixture is allowed to cool and the extract is filtered using whatman filter paper no.1 . The resultant filtrate is collected and used as plant extract and for nanoparticle preparation.

Cleaning and Drying gives the plant material a thorough cleaning to get rid of any pollutants and dirt. Drying the substance lowers its moisture content, aiding in effective extraction and preservation. Size Reduction is to improve the extraction surface area, chop or grind the dried plant material into smaller bits. The extraction efficiency of bioactive chemicals is improved by this procedure. To extract bioactive substances, use the proper extraction technique: Solvent Extraction is done depending on the solubility of the substances, extract them using solvents. Ultrasonic-Assisted Extraction: Uses ultrasonic waves to increase extraction efficiency. Utilizing microwave energy to aid in extraction is known as microwave-assisted extraction. Supercritical Fluid Extraction: High pressure extraction using supercritical fluids. After extraction, pass the extract through a filter to get rid of any solid debris or particles, leaving behind a transparent solution of the solvent-dissolved bioactive chemicals. To eliminate the solvent and get a concentrated extract, concentrate the filtered extract using techniques like freeze-drying or rotary evaporation. Characterization: Measure the quantity of bioactive chemicals in the concentrated extract and make sure the desired substances are pre.



Figure 1 : POWDERED PLANT EXTRACT

Plant extract as precursor of synthesis of nanoparticles :

Utilize the concentrated plant extract as a starting point for creating nanoparticles.

Green Synthesis: Mix the extract with a suitable metal salt solution (zinc sulphate, to make zinc oxide nanoparticles), then use the right reducing agents to reduce the mixture to nanoparticle size. Nanoparticle Characterization: Verify the size, shape, stability, and bioactivity of the produced nanoparticles by characterizing them.

Use the plant extract-based nanoparticles for antibacterial agents, medication delivery, and agricultural applications, among other uses.

In order to produce plant extract-based nanoparticles with the appropriate qualities for particular applications, each stage of this procedure is essential. The properties and bioactivity of the produced nanoparticles are strongly influenced by the selection of plant material and extraction technique.

Synthesis of nanoparticles:

Owing to the wide range of applications offered by nanoparticles in different fields of science and technology, different approaches have been developed for the synthesis of nanoparticles. In this study we prepared nanoparticles. The Ion diffusion method controlled by ion exchange membrane with agar hydrogel template is a novel approach for preparing inorganic nanomaterials, which has the advantages of mild reaction conditions, easy operation, energy saving and environmental friendliness.

Synthesis of Zinc Oxide Nanoparticles:

For the preparation of zinc oxide nanoparticles, 0.034 grams of zinc sulfate was mixed with 70 ml of distilled water. After the dissolution was completed, the zinc sulfate solution was mixed with 30 ml of commelina benghalensis extract. The zinc sulfate solution and the plant extract was kept in an orbital shaker for 48 hours. The synthesized nanoparticles were subjected to UV readings for confirmation of nanoparticles.

After 48 hrs, the zinc oxide nanoparticles are prepared under centrifuge of 8000 rpm for 10 minutes after completing the centrifugation process. Control reaction conditions such as temperature and pH to optimize nanoparticle size and stability. The pellets formed were collected and used for further biomedical applications.



Figure 2: preparation of plant extract and ZnONP solution

Preparation of *Commelina benghalensis* Extract:

Follow the steps mentioned earlier to prepare an extract from *Commelina benghalensis*. Choose an extraction solvent that is compatible with subsequent nanoparticle synthesis methods (e.g., water, ethanol).

Concentrate the extract to obtain a concentrated solution of bioactive compounds from *Commelina benghalensis*.

Filtering the plant powder from solution

After addition of commelina benghalensis plant extract to the solution, filter the plant powder.

Combining *Commelina benghalensis* Extract with Zinc Oxide Nanoparticles

Once the zinc oxide nanoparticles are synthesized and stabilized, allow the solution to cool to room temperature. Gradually add the concentrated *Commelina benghalensis* extract to the zinc oxide nanoparticle

solution under gentle stirring. Adjust the pH if necessary to ensure stability and compatibility between the plant extract and nanoparticles.



Figure: 3 Zinc nano particle and plant extract solution

Antimicrobial activity

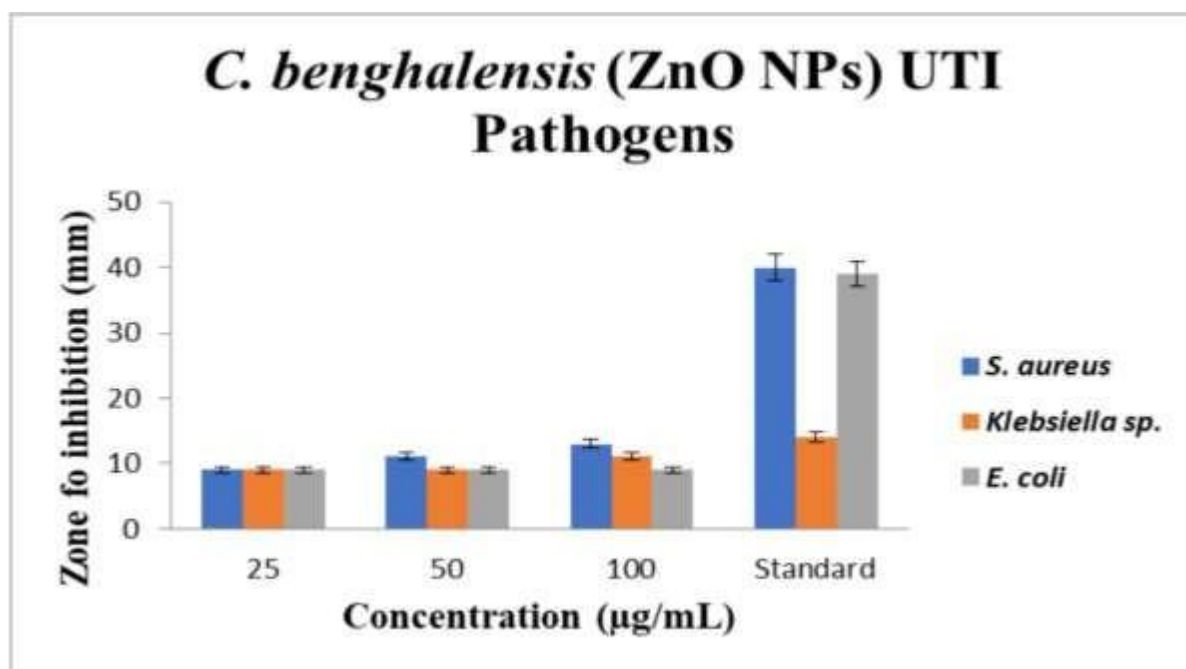
The antimicrobial activity of the green synthesized zinc oxide nanoparticles was evaluated using the agar well diffusion technique. Mueller Hinton agar plates were prepared and sterilized using an autoclave at 121°C for 15- 20 minutes. After sterilization, the medium was poured onto the surface of sterile Petri plates and allowed to cool to room temperature. The bacterial suspension (*escherichia coli*, *klebsiella sp*, *staphylococcus aureus*) was spread evenly onto the agar plates using sterile cotton swabs. Wells of 9 mm diameter were created in the agar plates using a sterile polystyrene tip. The wells were then filled with different concentrations (25, 50, 100 µg/mL) of ZnONPs. An antibiotic (e.g. Bacteria-Amoxyrite, Fungi-Fluconazole) was used as a standard. The plates were incubated at 37°C for 24 hours and 48 hours for fungal cultures. The antimicrobial activity was evaluated by measuring the diameter of the inhibition zone surrounding the wells. The diameter of the zone of inhibition was measured using a ruler and recorded in millimeters (mm) and the zone of inhibition was calculated.



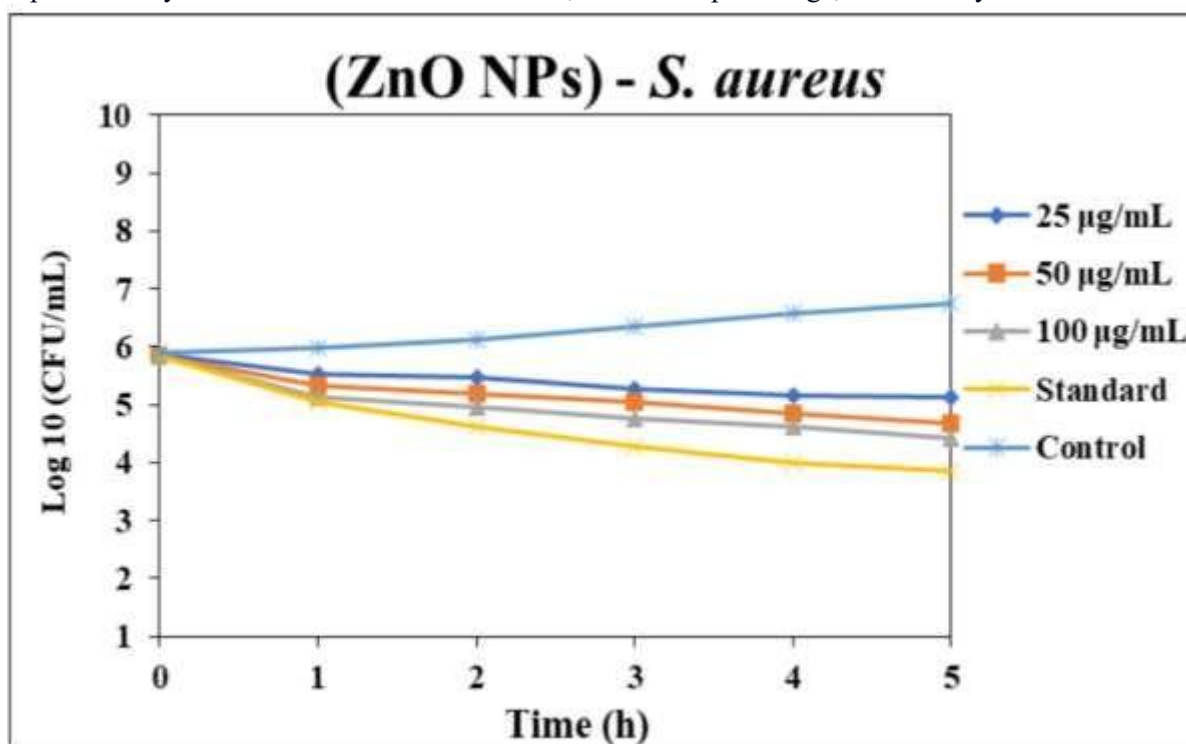
Figure 4: Plate for antimicrobial activity showing zone of inhibition

	25	50	100	Standard
S. aureus	9	11	13	40
Klebsiella sp.	9	9	11	14
E. coli	9	9	9	39

Table :1 Showing Concentration with zone of inhibition

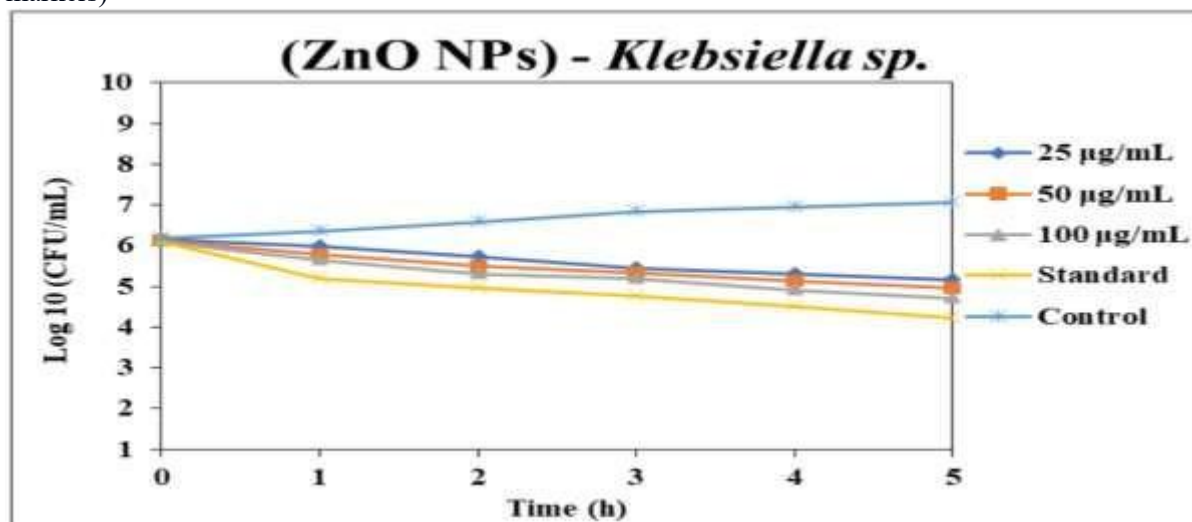


Graph 1: shows the zone of inhibition (measured in mm) for three different pathogenic bacteria *S.aureus* ,*Klebsiella sp* ,*E.coli* at various concentrations of the *C. benghalensis* (ZnO NPs) treatment. The x-axis represents the concentration of the treatment in µg/mL, with four categories: 25, 50, 100, and Standard. The y-axis shows the zone of inhibition in millimeters, ranging from 0 to 50 mm. Each bacterial species is represented by a different color: *S. aureus*: Blue ,*Klebsiella sp.*: Orange,*E. coli*: Gray

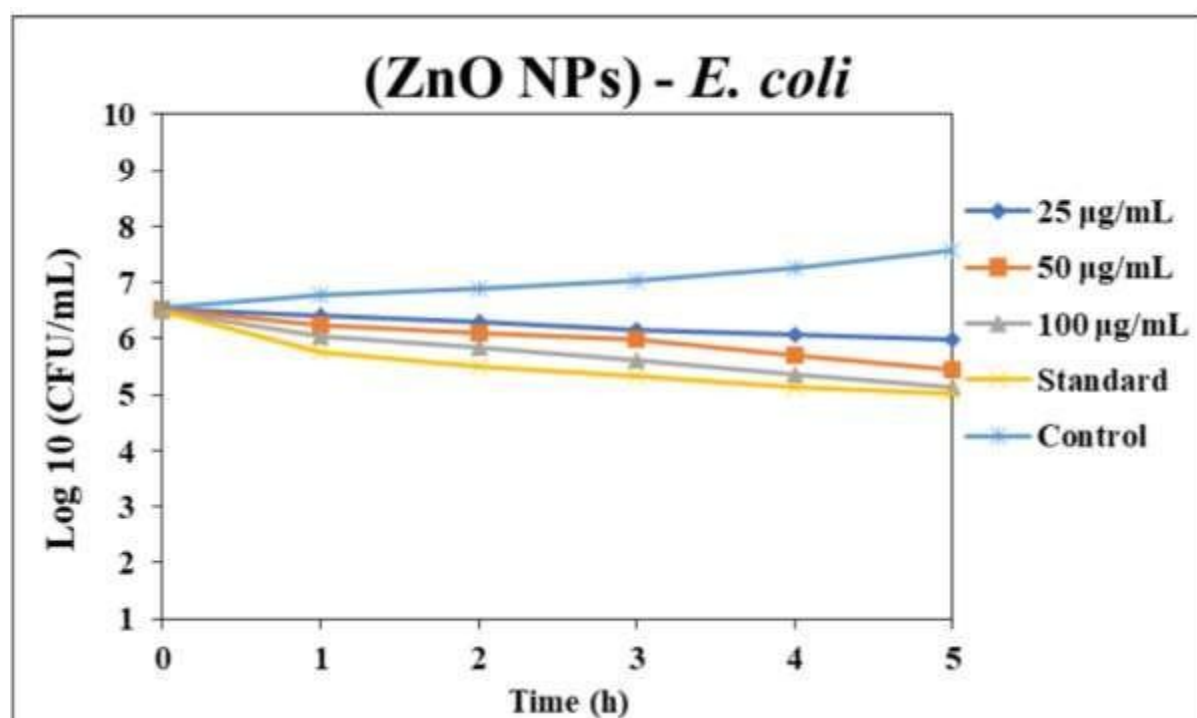


Graph 2- depicts the effect of different concentrations of Zinc Oxide Nanoparticles (ZnO NPs) on the growth of *Staphylococcus aureus* (*S. aureus*) bacteria over time. X-axis: Represents time in hours, ranging from 0 to 5 hours. Y-axis: Represents Log₁₀ (CFU/mL), which stands for the logarithm (base 10) of Colony Forming Units per milliliter. This is a measure of bacterial concentration. The y-axis ranges from 1 to 10. The graph includes five different conditions, each represented by a different colored line: 25 µg/mL ZnO NPs (blue line with diamond markers) 50 µg/mL ZnO NPs (orange line with square markers) 100

$\mu\text{g/mL}$ ZnO NPs (gray line with triangle markers) Standard (yellow line) Control (light blue line with star markers)



Graph 3 displays the effect of different concentrations of zinc oxide nanoparticles (ZnO NPs) on the growth of *Klebsiella sp.* bacteria over time. The x-axis represents Time (h) from 0 to 5 hours, while the y-axis shows Log 10 (CFU/mL) ranging from 1 to 10. CFU stands for Colony Forming Units, a measure of viable bacterial cells. The graph includes five different conditions, represented by different color - 25 $\mu\text{g/mL}$ (blue line with diamond markers) 50 $\mu\text{g/mL}$ (orange line with square markers) 100 $\mu\text{g/mL}$ (gray line with triangle markers) Standard (yellow line with x markers) Control (light blue line with asterisk markers)



Graph 4 - illustrates the effect of different concentrations of zinc oxide nanoparticles (ZnO NPs) on the growth of *Escherichia coli* (*E. coli*) bacteria over time. X-axis: Represents time in hours, ranging from 0 to 5 hours. Y-axis: Shows Log10 (CFU/mL), which stands for the logarithm (base 10) of Colony Forming Units per milliliter. This is a measure of bacterial concentration. The y-axis ranges from 1 to 10. The graph contains five different lines, each representing a different experimental condition: Blue line with diamond markers: 25 $\mu\text{g/mL}$ of ZnO NPs, Orange line with square markers: 50 $\mu\text{g/mL}$ of ZnO NPs, Gray line with triangle markers: 100 $\mu\text{g/mL}$ of ZnO NPs, Yellow line with X markers: Standard (likely a standard antibiotic or treatment), Light blue line with asterisk markers: Control (no treatment)

DISCUSSION

Graph 1 shows the zone of inhibition (measured in mm) for three different pathogenic bacteria *S.aureus*, *Klebsiella* sp, *E.coli* at various concentrations of the *C. benghalensis* (ZnO NPs) treatment. The x-axis represents the concentration of the treatment in ug/mL, with four categories: 25, 50, 100, and Standard. The y-axis shows the zone of inhibition in millimeters, ranging from 0 to 50 mm. Each bacterial species is represented by a different color: *S. aureus*: Blue ,*Klebsiella* sp.: Orange,*E. coli*: Gray . The graph demonstrates the following

At 25 ug/mL concentration, all three bacteria show similar zones of inhibition, around 9-10 mm. At 50 ug/mL, there's a slight increase in the zone of inhibition for all bacteria, with *S. aureus* showing the highest at about 11 mm. At 100 ug/mL, *S. aureus* shows the largest zone of inhibition (about 13 mm), followed by *Klebsiella* sp., and then *E. coli*. The "Standard" category shows much larger zones of inhibition for *S. aureus* and *E. coli* (both around 40 mm), while *Klebsiella* sp. has a lower value (about 15 mm). This graph effectively compares the antibacterial efficacy of *C. benghalensis* (ZnO NPs) against these three UTI pathogens at different concentrations, as well as against a standard treatment.

Based on the information in the graph, we can make several observations like Concentration-dependent effect: The zone of inhibition generally increases with higher concentrations of *C. benghalensis* (ZnO NPs) for all three bacteria. Differential sensitivity: The three bacterial species show different levels of sensitivity to the treatment ,*S.aureus* appears to be the most sensitive, showing the largest zones of inhibition at higher concentrations,*Klebsiella* sp. and *E. coli* show similar patterns, but with slightly smaller zones of inhibition compared to *S. aureus*. Standard comparison: The "Standard" treatment (likely a known antibiotic) shows much larger zones of inhibition for *S. aureus* and *E. coli* compared to the *C. benghalensis* (ZnO NPs) treatment. However, for *Klebsiella* sp., the difference is less pronounced. Potential effectiveness: The results suggest that *C. benghalensis* (ZnO NPs) has antibacterial properties against these UTI pathogens, with increasing effectiveness at high concentrations.

Graph 2 depicts the effect of different concentrations of Zinc Oxide Nanoparticles (ZnONPs) on the growth of *Staphylococcus aureus* (*S. aureus*) bacteria over time. X-axis: Represents time in hours, ranging from 0 to 5 hours. Y-axis: Represents Log₁₀ (CFU/mL), which stands for the logarithm (base 10) of Colony Forming Units per milliliter. This is a measure of bacterial concentration. The y-axis ranges from 1 to 10. The graph includes five different conditions, each represented by a different colored line: 25 ug/mL ZnO NPs (blue line with diamond markers), 50 ug/mL ZnO NPs (orange line with square markers), 100 ug/mL ZnO NPs (gray line with triangle markers), Standard (yellow line), Control (light blue line with star markers). All lines start at approximately the same point (around 6 on the y-axis) at 0 hours. The Control line shows an increase over time, indicating bacterial growth in the absence of ZnO NPs. The Standard line and all ZnO NP concentration lines show a decrease over time, suggesting an inhibitory effect on bacterial growth. The higher concentrations of ZnO NPs (50 and 100 ug/mL) show a more pronounced decrease in bacterial concentration compared to the lower concentration (25 ug/mL). The Standard line shows the steepest decline, indicating it might be a known antibiotic or treatment used for comparison. This graph effectively illustrates the dose-dependent antibacterial effect of ZnO nanoparticles on *S. aureus* over a 5-hour period, with higher concentrations generally showing greater inhibition of bacterial growth.

Graph 3 displays the effect of different concentrations of zinc oxide nanoparticles (ZnONPs) on the growth of *Klebsiella* sp. bacteria over time. The x-axis represents Time (h) from 0 to 5 hours, while the y-axis shows Log₁₀ (CFU/mL) ranging from 1 to 10. CFU stands for Colony Forming Units, a measure of viable bacterial cells. The graph includes five different conditions, represented by different colored lines and symbols 25 ug/mL (blue line with diamond markers), 50 ug/mL (orange line with square markers), 100 ug/mL (gray line with triangle markers), Standard (yellow line with x markers), Control (light blue line with asterisk markers) . All lines start at around 6 Log₁₀ CFU/mL at 0 hours. Over the 5-hour period.

The Control shows a steady increase, reaching about 7 Log₁₀ CFU/mL by 5 hours. The Standard and all ZnO NP concentrations show a decrease in bacterial growth. The Standard treatment shows the steepest

decline. Among the ZnO NP concentrations, 100 µg/mL appears to be most effective in reducing bacterial growth, followed by 50 µg/mL and then 25 µg/mL.

Graph 4 illustrates the effect of different concentrations of zinc oxide nanoparticles (ZnO NPs) on *Escherichia coli* (E. coli) over time. The x-axis represents Time (h) ranging from 0 to 5 hours, while the y-axis shows Log₁₀ (CFU/mL), with values ranging from 1 to 10. The graph contains five different lines, each representing a different concentration or condition: 25 µg/mL (blue line with diamond markers), 50 µg/mL (orange line with square markers), 100 µg/mL (gray line with triangle markers), Standard (yellow line with x markers), Control (light blue line with asterisk markers). All lines start at approximately the same point around 6.5 Log₁₀ (CFU/mL) at 0 hours. Over the course of 5 hours: The Control line shows an increase, reaching about 7.5 Log₁₀ (CFU/mL) by the end. The 25 µg/mL, 50 µg/mL, and 100 µg/mL lines all show a gradual decrease, with the 100 µg/mL line decreasing the most. The Standard line shows the steepest decrease, ending at about 5 Log₁₀ (CFU/mL). This graph demonstrates that higher concentrations of ZnO NPs lead to greater reductions in E. coli populations over time, while the control Ahmad et al. (2023) investigated the antimicrobial efficacy of biogenic zinc oxide nanoparticles (ZnO NPs) made from *Mentha piperita* extract. Bacterial activity was assessed through agar well diffusion method. The biogenic ZnO NPs showed a significantly larger inhibition zone than standard antibiotics on two species of bacteria *Proteus mirabilis* and *Pseudomonas aeruginosa*, and a fungus, *Candida albicans*. Additionally, the ZnO NPs alone showed stronger antimicrobial ability than any other combination of that were the plant extract with ZnO NPs or the plant extract alone.

In another study (Biologically synthesized ZnO NPs...), they reported that biologically synthesis of ZnO nanoparticles has a strong effect against ESBL (Extended Spectrum Beta-Lactamase) producing bacterial strains. This is an important finding, as to our knowledge, no previous studies have reported the antibacterial effects of metal oxide nanoparticles, such as ZnO NPs against ESBL producing Gram-negative bacteria. The antibacterial effects, were attributed to the nanoparticles ability to potentially generate reactive oxygen species (ROS) and the impairment of bacterial cell wall, to inhibit growth, which could be the first-ever evidence of this kind.

Jacob Inbaneson et al. (2011) investigated the antibacterial effectiveness of silver nanoparticles (AgNPs) against microorganisms from urinary tract infections (UTIs). The researchers isolated 32 bacterial species from midstream urine collecting 50 samples (25 male and 25 female individuals) from Thondi, Ramanathapuram District in Tamil Nadu, India. *Escherichia coli* was the most frequently observed isolate at 47% followed by *Pseudomonas aeruginosa* (22%), *Klebsiella pneumoniae* (19%), *Enterobacter* sp. (6%), *Proteus morganii* (3%), and *Staphylococcus aureus* (3%). Evaluating the measures of propellant, they found that using the disc diffusion method AgNPs showed maximum activity against *P. aeruginosa* (11 ± 0.58 mm) and *Enterobacter* sp. (8 ± 0.49 mm) at a concentration of 20 µg/disc using results comparable to the standard antibiotics, kanamycin and tetracycline. In contrast, *K. pneumoniae*, *E. coli*, *P. morganii*, and *S. aureus* showed resistance to all concentrations tested.

In the study titled "Phyto-mediated synthesis of zinc oxide...", researchers synthesized ZnO nanoparticles using leaf extract from the medicinal plant *Berberis aristata*. The nanoparticles were characterized using UV-Visible spectroscopy, X-ray diffraction (XRD), scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX), and dynamic light scattering (DLS), confirming the formation of nanoscale particles. The ZnO NPs exhibited antimicrobial activity against various pathogens, including *E. coli*, *S. aureus*, *K. pneumoniae*, *Bacillus subtilis*, *Bacillus cereus*, and *Serratia marcescens*. However, they showed no activity against *Proteus* and *Salmonella Typhi*. Additionally, the ZnO NPs demonstrated moderate antioxidant properties, highlighting their potential use as antimicrobial and antioxidant agents, especially for treating UTIs.

Another study (Green synthesized ZnO NPs as effective...) focused on the green synthesis of ZnO nanoparticles from *Limonia acidissima*. These nanoparticles were evaluated for their antibacterial efficacies against biofilm-forming urinary tract infection pathogens; namely, *Salmonella paratyphi*, *Shigella*, *Streptococcus*, *Staphylococcus*, and *K. pneumoniae*, and confirmation of their synthesis was determined by UV-Visible spectrophotometry and FTIR, which signaled the presence of biomolecules aimed in nanoparticle stabilization. Scanning electron microscopy gave detailed information on

morphology and sizes. The ZnO NPs displayed significant antimicrobial activity in a variety of in vitro assays indicating their potential in developing novel therapeutic approaches against biofilm-associated urinary tract infections.

In an experimental study by Torabi (2017), urine samples containing multidrug resistant *E. coli* underwent treatment of colloidal ZnO nanoparticles diameter size of 20 nm and spherical in shape at a concentration of 200 ppm; antibacterial activity was determined from both well and disc diffusion on nutrient agar and LB media, Adult *E. coli* was incubated at 37°C for 72 hours. The results indicated *E. coli* had highest levels of resistance to cefazolin and cefixime, while ZnO NPs exhibited effective antibacterial activity. In conclusion, ZnO NPs of this specification may provide potential treatment of urinary tract infections caused by resistant strains of *E. coli*.

Kalpna and Devi Rajeswari (2018) described that green synthesis of ZnO nanoparticles is environmentally friendly and does not generate toxic byproducts, as in the case with physical and chemical methods. The application of ZnO NPs in the biomedical field is being rapidly developed and is being investigated in areas, such as, bioimaging, drug delivery, biosensing, and gene delivery. ZnO NPs exhibit promising antimicrobial activity and are considered a possible substitute for antibiotics in treatment of multidrug-resistant microorganisms.

Bharathy et al. (2024), used a green method to synthesize ZnO nanoparticles using the millet plant, *Echinochloa esculenta*. The NPs were characterized using UV, FTIR, XRD, SEM, and EDX to confirm the formation, morphology, and composition of the nanoparticles synthesized. The synthesized particles were found to have a spherical shape with an average size of 23.38 nm. The particles contained 73.33 weight % zinc and 26.77 % oxygen. When tested against five common UTI pathogens, the ZnO NPs were found to have a greater inhibition zone than zinc acetate and the extract of the plant. The plants had the strongest inhibitory effect against *Staphylococcus aureus* suggesting that these nanoparticles would be effective for UTI treatment strategies.

Because of their improved biocompatibility, antimicrobial effectiveness, and environmentally friendly production, green-synthesised nanoparticles have great potential for use in biomedical applications. In an animal model, Sreenivasagan et al. (2023) showed that titanium mini-implants coated with silver nanoparticles that were green-synthesised showed better biological acceptance and less toxicity than uncoated implants. The research emphasizes how these nanoformulations can reduce tissue reactions while simultaneously offering long-lasting and potent antimicrobial protection, which will increase the clinical efficacy of implant-based treatments.

Garapati et al. (2022) assessed the cytotoxicity and biosafety of green-synthesised nanoparticles, particularly lycopene-mediated silver nanoparticles, in zebrafish embryonic development. Their research demonstrated that, while retaining strong biological activity, plant-mediated nanoparticle synthesis provides a biocompatible and environmentally responsible substitute for traditional chemical processes. Crucially, the study underlined that in order to guarantee safe biomedical applications, toxicological profiles of green-synthesised nanoparticles in biological systems must be evaluated.

Future scope:

UTIs are often exacerbated by bacterial biofilms that adhere to urinary tract surfaces, making them resistant to antibiotics. ZnONPs have shown effectiveness in disrupting biofilms in other contexts. Investigating their ability to prevent biofilm formation or to disperse existing biofilms in UTIs could be a promising avenue for research. Assessing the biocompatibility and safety of *Commelina benghalensis* mediated ZnONPs is crucial for their application in UTI treatment. Future studies should focus on understanding how these nanoparticles interact with human cells and tissues, ensuring they are safe for therapeutic use. Exploring potential synergies between ZnONPs and conventional antibiotics could enhance their efficacy against antibiotic-resistant UTI pathogens. Combination therapies may reduce the concentration of antibiotics needed, potentially lowering the risk of antibiotic resistance development. Developing targeted delivery systems for ZnONPs to the urinary tract could improve their efficacy while minimizing systemic side effects. This could involve encapsulating the nanoparticles in biocompatible materials or designing coatings that enhance their specificity to UTI-causing bacteria. Understanding the environmental impact of ZnONPs synthesized from natural sources like

Commelina benghalensis is crucial. Research should evaluate their degradation pathways and potential ecological consequences to ensure responsible use.

CONCLUSION: This study aimed to demonstrate the effects of *Commelina benghalensis* mediated zinc oxide nanoparticles against UTI pathogens. At 25 µg/mL concentration, all three bacteria show similar zones of inhibition, around 9-10 mm. At 50 µg/mL, there's a slight increase in the zone of inhibition for all bacteria, with *S. aureus* showing the highest at about 11 mm. At 100 µg/mL, *S. aureus* shows the largest zone of inhibition (about 13 mm), followed by *Klebsiella* sp., and then *E. coli*. The zone of inhibition generally increases with higher concentrations of *C. benghalensis* (ZnO NPs) for all three bacteria. The "Standard" category shows much larger zones of inhibition for *S. aureus* and *E. coli* (both around 40 mm), while *Klebsiella* sp. has a lower value (about 15 mm). The three bacterial species show different levels of sensitivity to the treatment, *S. aureus* appears to be the most sensitive, showing the largest zones of inhibition at higher concentrations, *Klebsiella* sp. and *E. coli* show similar patterns, but with slightly smaller zones of inhibition compared to *S. aureus*. The "Standard" treatment (likely a known antibiotic) shows much larger zones of inhibition for *S. aureus* and *E. coli* compared to the *C. benghalensis* (ZnO NPs) treatment. However, for *Klebsiella* sp., the difference is less pronounced. Potential effectiveness: The results suggest that *C. benghalensis* (ZnO NPs) has antibacterial properties against these UTI pathogens, with increasing effectiveness at high concentrations. Time (vs) growth data gives the conclusion that concentration of zinc oxide nanoparticles is inversely proportional to the growth rate of bacteria. Results display the synthesized ZnO NPs to fall in an important class of potential antibacterial, antioxidant agents and effective against urinary tract infections. The enhanced antibacterial properties points to useful applications in UTI treatment. Further research may focus on increasing the effectiveness of nano formulation.

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